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Research Article

DESIGN OF PVA & XANTHAN BASED HYDROGELS INCORPORATED WITH SILVER NANOPARTICLES

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ABSTRACT

Hydrogels have been widely recognized for their biocompatibility and high water content in biomedical applications. Aiming to harness nanosilver's antimicrobial properties for wound healing, this study develops and evaluates xanthan gum and polyvinyl alcohol (PVA) based hydrogels incorporating nanosilver. By freezing and thawing the hydrogels, nanosilver is distributed uniformly throughout the matrix. FTIR was used to confirm the presence of functional groups and interactions between xanthan, PVA, and nanosilver in the hydrogels. Observations of the surface morphology and distribution of nanosilver particles were conducted using scanning electron microscopy (SEM). Standard microbiological techniques were used to assess the antimicrobial efficacy of nanosilver-loaded hydrogels against common wound pathogens, including Staphylococcus aureus and Escherichia coli. Significant inhibition zones were observed in treated cultures compared to controls, indicating strong antibacterial activity. In conclusion, the xanthan/PVA hydrogels containing nanosilver show promising properties for application as antimicrobial wound dressings, because they possess both effective bacterial inhibition and favorable physical and biological properties.

Keywords:- Xanthan Gum, Polyvinyl Alcohol, Hydrogels, Nanosilver, Antimicrobial, Wound Healing, Biocompatibility.

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INTRODUCTION

Hydrogels are three-dimensional (3D) materials with the ability to absorb large amounts of water while maintaining their dimensional stability. The 3D integrity of hydrogels in their swollen state is maintained either by physical or chemical crosslinking. Lower interfacial tension, soft and tissue-like physical properties, higher permeability to undersized molecules and release of entrapped molecules in a controlled manner made hydrogels to be explored in different biomedical fields. In the absence of crosslinking points, hydrophilic linear polymer chains dissolve in water due to the thermodynamic compatibility of the polymer chains and water [1, 2]. The presence of chemical or physical crosslinking points within the network maintains the three-dimensional integrity of hydrogels in their swollen state. In chemically cross-linked hydrogels, the linear polymer chains are covalently bonded with each other via crosslinking agents [3, 4].

Classification of Hydrogels

Hydrogels are mainly classified as natural or

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synthetic according to their origin. Table- 1 lists some of the natural polymers and synthetic monomers from which hydrogels can be prepared.

The network stability of hydrogels in their swollen state is due to the presence of either chemical or physical crosslinking. Chemically cross linked hydrogels are also known as thermosetting hydrogels or permanent gels. Moreover the crosslinking agents used to prepare hydrogels are highly toxic and the residues must be completely removed before their use as biomaterials [9]. Physically cross linked hydrogels, on the other hand, maintain their physical stability due to the presence of reversible physical junction domains associated with hydrogen bonding, hydrophobic interaction, chain entanglements, crystallinity, and/or ionic complexation [5, 6]. Physically cross-linked hydrogels are also known as thermoplastic hydrogels or temporary gels.

Preparation of Hydrogels

There are two suggested mechanisms behind physical hydrogel formation, the first one being the gelation of nanofibrous peptide assemblies, usually observed for oligopeptide precursors. The precursors self-assemble into fibers, tapes, tubes, or ribbons that entangle to form non-covalent cross-links. The second mechanism involves non-covalent interactions of cross-linked domains that are separated by water-soluble linkers, and this is usually observed in longer multi-domain structures.

Physically crosslinked hydrogels can be prepared by different methods depending on the nature of the crosslink involved. Polyvinyl alcohol hydrogels are usually produced by the freeze-thawed technique. In this, the solution is frozen for a few hours, then thawed at room temperature, and the cycle is repeated until a strong and stable hydrogel is formed.

Isostatic Ultra High Pressure (IUHP)

Here the suspension of natural biopolymers like starch, are subjected to ultrahigh pressure of 300-700 MPa for 5 or 20 min in a chamber which brings about changes in the morphology of the polymer (i.e. HPMCization of starch molecules occur). It is different from heat-induced HPMCization where a change in ordered state of polymer occurs. Usually the temperature within the chamber varies from 40 to 52°C [7].

Use of Cross Linkers

Since hydrogels are the polymers which swell in presence of water and they entrap drug within their pores; therefore, to impart sufficient mechanical strength to these polymers, cross linkers are incorporated like glutaraldehyde, calcium chloride and oxidized konjac glucomannan (DAK) [8].

Use of Water and Critical Conditions of Drying

Aerogels of carbon have been prepared by super critically controlling the drying conditions. Aerogels of resorcinol formaldehyde hydrogels have also been prepared by using water as solvent and sodium carbonate as pH regulator [9].

Use of Gelling Agents

Gelling agents like glycophosphate, 1-2 propanediol, glycerol, trehalose, mannitol, etc, have been used in formation of hydrogels. Usually the problem of turbidity and presence of negative charged moieties which are associated with this method pose problem of interaction with the drug [10].

Use of Irradiation and Freeze Thawing

Hydrogels prepared by chemical methods (i.e. use of crosslinkers, gelling agents or reaction initiators) are having problems of removal of residue or unnecessary charged moieties present. Irradiation method is suitable and convenient but the processing is costly. The mechanical strength of such hydrogels is less. However with freeze thawing method, the hydrogels so formed have sufficient mechanical strength and stability but are opaque in appearance with a little swelling capacity. However, hydrogels prepared by microwave irradiation are more porous than conventional methods [11].

Water in Hydrogels

Swelling behaviour of hydrogel systems is an important parameter governing their applications specifically in pharmaceutical, ophthalmology and tissue engineering. The presence of water at the surface of hydrogels reduces the interfacial free energy in a physiological environment and thus improves their biological properties. The final water content of hydrogels depends on both kinetics and thermodynamics parameters. During the swelling process, the first water molecules hydrate the most polar, hydrophilic groups, and this portion of water is called ‘primary bound water’. As the hydration of polar and hydrophilic groups is completed, the network swells, and exposes hydrophobic groups, which start interacting with water through hydrophobic interaction called secondary bound water molecules. Together, primary and secondary bound water molecules are often called the total bound water [12].

Applications of Hydrogels

- Wound healing
- In tissue engineering
- For gene delivery
- Colon specific drug delivery
- Cosmetology

- Topical drug delivery
- Protein drug delivery
- Miscellaneous applications [13 - 18]

Pharmaceutical Applications

Hydrogels have been attempted extensively to achieve ideal drug delivery systems with desirable therapeutic features. The unique attractive physicochemical and biological characteristics of hydrogels, along with their huge diversity, collectively, have led to considerable attention to these polymeric materials as excellent candidates for delivery systems of therapeutic agents. Pharmaceutical hydrogels have been categorized according to a variety of criteria mainly including, rout of administration.

Silver Nanoparticles

Resistance in human pathogens is a big challenge in fields like pharmaceutical and biomedicine. Antibiotic resistance profiles lead to fear about the emergence and re- emergence of multidrug-resistant (MDR) pathogens and parasites. Once an individual is infected with MDR bacteria, it is not possible to cure easily and he/she has to spend more time in the hospital and requires a multiple treatment of broad- spectrum antibiotics, which are less effective, more toxic and more expensive. Therefore, development of or modification in antimicrobial compounds to improve bactericidal potential is a priority area of research in this modern era. Nanotechnology provides a good platform to modify and develop the important properties of metal in the form of nanoparticles having promising applications in diagnostics, biomarkers, cell labelling, contrast agents for biological imaging, antimicrobial agents, drug delivery systems and nanodrugs for treatment of various diseases.

Silver-Based Antimicrobials

Silver is a basic, rare and naturally occurring element, which is slightly harder than gold, very ductile and malleable, having the highest electrical and thermal conductivity with minimum contact resistance within all metals. Various types of silver compounds that are used as antimicrobials from ancient times include silver nitrate, silver sulfadiazine, silver zeolite, silver powder, silver oxide, silver chloride and silver cadmium powder. Since the ancient time, silver nanoparticles have emerged as antimicrobial agents owing to their high surface- area-to-volume ratio and the unique chemical and physical properties.

Silver and Wound Infection

The use of topical chemotherapy is fundamental to prevent infections in deep and superficial burns or extensive intermediary burns. Increasingly, antibiotics, due to widespread indiscriminate prescription, are becoming less effective as pathogens are becoming more resistant to their action. Silver may be a useful prophylactic or therapeutic agent for the prevention of wound colonization by organisms that impede healing, including antibiotic-resistant bacteria.

Preparation Methods for Silver Nanoparticles

- Physical Approaches
- Chemical Approaches
- Biological Approaches
- Electrochemical Method

Silver Nanoparticles Applications

In the past, silver was used for a variety of clinical conditions including epilepsy, venereal infections, acnes and leg ulcers. Silver foil was applied to surgical wounds for improved healing and reduced post-operative infections, while silver and lunar caustic (pencil containing silver nitrate mitigated with potassium nitrate) was used for wart removal and ulcer debridement.

- Diagnostic Applications
- Antibacterial Applications
- Conductive Applications
- Optical Applications

MATERIALS AND METHODS

Materials Used

Xanthan, Poly vinyl alcohol, Silver nitrate, Sodium citrate, Glycerol, Hydrochloric acid, Glutaraldehyde, Peptone, Agar, Yeast extract, Glucose, Beef extract.

Equipment Used

Mechanical stirrer, Magnetic stirrer, Hot plate, UV-visible spectrophotometer, FTIR, Autoclave Incubator, SEM, Digital weighing machine.

Experimental Design

Preparation of Hydrogels Incorporated with Silver Nanoparticles (GSN)

Weigh accurately 17 mg of AgNO3 and dissolved in a 100 ml of distilled water in a volumetric flask to prepare 0.001M of silver nitrate solution. Weigh accurately 0.13 g of sodium citrate and dissolved in a 50 ml of distilled water in a volumetric flask to prepare 10mM of sodium citrate solution. 100 ml of 0.001M AgNO3 was heated to boiling point. To the heated solution 10mM sodium citrate was added drop by drop with vigorous stirring until a bright yellowish color is obtained. The appearance of color indicates the formation of silver nanoparticles. The resulting solution was then removed from the heating element and stirred until cooled to room temperature.

For the preparation of silver nanoparticles incorporated XANTHAN/PVA hydrogel sheets, 2.5gm of Xanthan was dissolved in 100ml of 10% aqueous solution of poly vinyl alcohol with continues stirring using mechanical stirrer at 100 ± 5 RPM at 70oC, until a clear solution is obtained. To this resulting solution 0.05 ml of hydrochloric acid was added drop wise with continues stirring. To this thick solution, glycerol was added drop wise until clear solution was obtained. The silver nanoparticles solution was added in a required quantity to the above solution and stirred for 15 minutes. The resulting mixture was poured in to OHP boats and kept for drying at room temperature for a period of two days. Same procedure was followed with various content of glycerol was added and it is labelled as GSN1, GSN2, GSN3 and GSN4.

Characterization of Silver Nanoparticles Incorporated Hydrogels (GSN)

Generally hydrogels are characterized for their morphology, swelling property and elasticity. Morphology is indicative of their porous structure. Swelling determines the release mechanism of the drug from the swollen polymeric mass while elasticity affects the mechanical strength of the network and determines the stability of these drug carriers. Some of the important features for characterization of hydrogels are as follows:

Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) is a useful technique for identifying chemical structure of a substance. It is based on the principle that the basic components of a substance, i.e. chemical bonds, usually can be excited and absorb infrared light at frequencies that are typical of the types of the chemical bonds. The resulting IR absorption spectrum represents a fingerprint of measured sample. This technique is widely used to investigate the structural arrangement in hydrogel by comparison with the starting materials.

FT-IR spectra of XANTHAN PVA, hydrogel sheet and silver nanoparticles incorporated XANTHAN/PVA hydrogel sheet were taken on a analytical technologies model 2202 spectrometer. Powdered samples were prepared into pellets with KBr.

UV-Visible Spectroscopy

UV-visible spectroscopy offers a relatively straightforward and effective way for quantitatively characterizing of both organic and inorganic compounds. It is one of the most widely used techniques for structural characterization of silver nanoparticles. It operates on the principle of absorption of photons that promotes the molecule to an excited state. It is an ideal technique for determining the electronic properties such as band gap of a material. Ag nanoparticles synthesized from chemical reduction method were characterized in a 'SHIMADZU' UV-VIS spectrophotometer. The scanning range for the samples was 200-800 nm. The spectrophotometer was equipped with "UV-Probe" software to record and analyse data. Base line correction of the spectrophotometer was carried out by using a blank reference.

Swelling and Absorption Capacity

The water absorption of XANTHAN/PVA hydrogel sheet was examined by water uptake capacity. Hydrogel sheet was cut into 1×1 cm 2 and note down their dry weight (W1). The swelling behavior of the hydrogel was measured by immersing them in distilled water at room temperature in Petridish. At different time intervals, remove hydrogel and the excess water on the surface of it was removed by blotting the surface with tissue paper and the weight was then recorded (W2). This process was repeated until the swelling equilibrium was achieved. The degree of swelling was calculated using the following equation:

Degree of swelling (%) = [(W2-W1)/ W1 x 100

Where, W1 is the weight of completely dried sample and W2 is the weight of swelled sample.

Scanning Electron Microscopy (SEM)

Scanning electron microscopy is an excellent tool for physical observation of morphological features of nanoparticles. This is a powerful technique widely used to capture the characteristic 'network' structure in hydrogels. Here, the hydrogel sheets were treated with liquid nitrogen to make them rigid and focused with the 'ZEISS' equipment to get the surface morphology and the particle size of the silver nanoparticles.

Antimicrobial Studies

The antimicrobial activity of silver nanoparticles is tested on two types of human pathogenic bacteria such as Escherichia coli and Staphylococcus aureus which were cultured on nutrient agar plates supplemented with different concentration of silver nanoparticles. The nutrient agar medium was autoclaved for 15 minutes. Then the medium was transferred to the sterilized petri plates and allowed to solidify. After solidification, under the laminar air flow chamber inoculate the bacteria present in the nutrient broth (100µl) in to the nutrient agar medium with the help of 'L' shaped spreader. After that Silver nanoparticles incorporated hydrogel sheets were placed over the plates in the form of disc shape and were incubated for 24 hr at 370C

RESULTS

Compatibility Studies by FTIR

The Fourier transform infrared (FTIR) spectra of XANTHAN, PVA and XANTHAN/PVA hydrogel sheet were recorded using KBr pellet method.

Swelling and Absorption Capacity

The degree of swelling was calculated using the following equation: Degree of swelling (%) = [(W2-W1)/ W1 x 100

Where, W1 is the weight of completely dried sample and W2 is the weight of swelled sample.

Characterization Studies of Prepared Silver Nanoparticles Visual Inspection

The formation of silver nanoparticles occurs after the reduction of aqueous silver salts with trisodium citrate (10mM) within duration of 10-20 minutes. Color change appears after the completion of reaction as the silver nanoparticles exhibit pale yellow color. When the reducing agent is added into aqueous silver salt in drop wise manner, growth of silver "seed nucleation" is controlled in Nano dimension. This change in color is an indication of the formation of silver nanoparticles

Ultraviolet-Visible Spectroscopy

The prepared silver nanoparticles showed an absorption bands at 397 nm and 420 nm, which is a typical absorption bands of spherical Ag nanoparticles. The absorption band in visible light region (350 nm – 550 nm, plasmon peak at 420 nm) is typical for silver nanoparticles

Scanning Electron Microscopy (SEM)

The SEM image of Silver nanoparticles synthesized by chemical reduction method by using 1mM AgNO3 concentration and 10mM of sodium citrate. It gave a clear image of Silver nanoparticles. The SEM image showing silver nanoparticles confirmed the development of silver nanostructures.

Figure 1: FTIR spectra of Xanthan

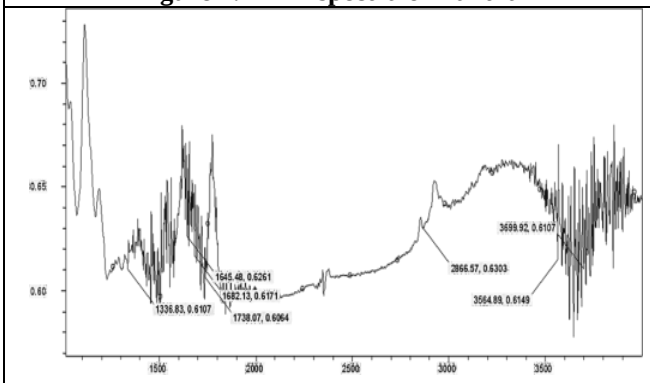


Figure 2: FTIR spectra of PVA

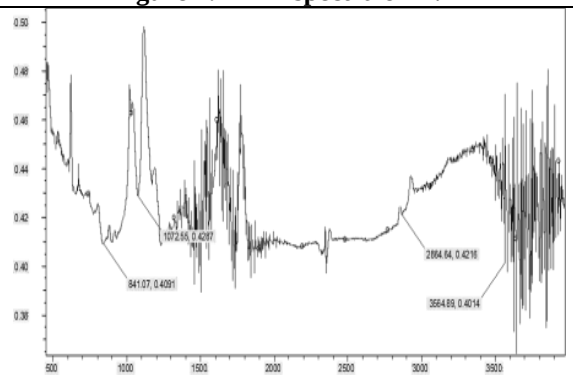


Figure 3: FTIR spectra of XANTHAN hydrogel sheet

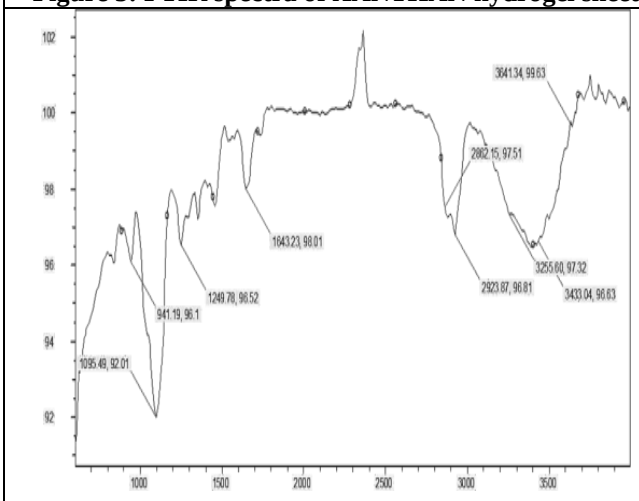


Figure 4: Prepared Hydrogel sheet

Figure 5: Swelling indices of Hydrogels

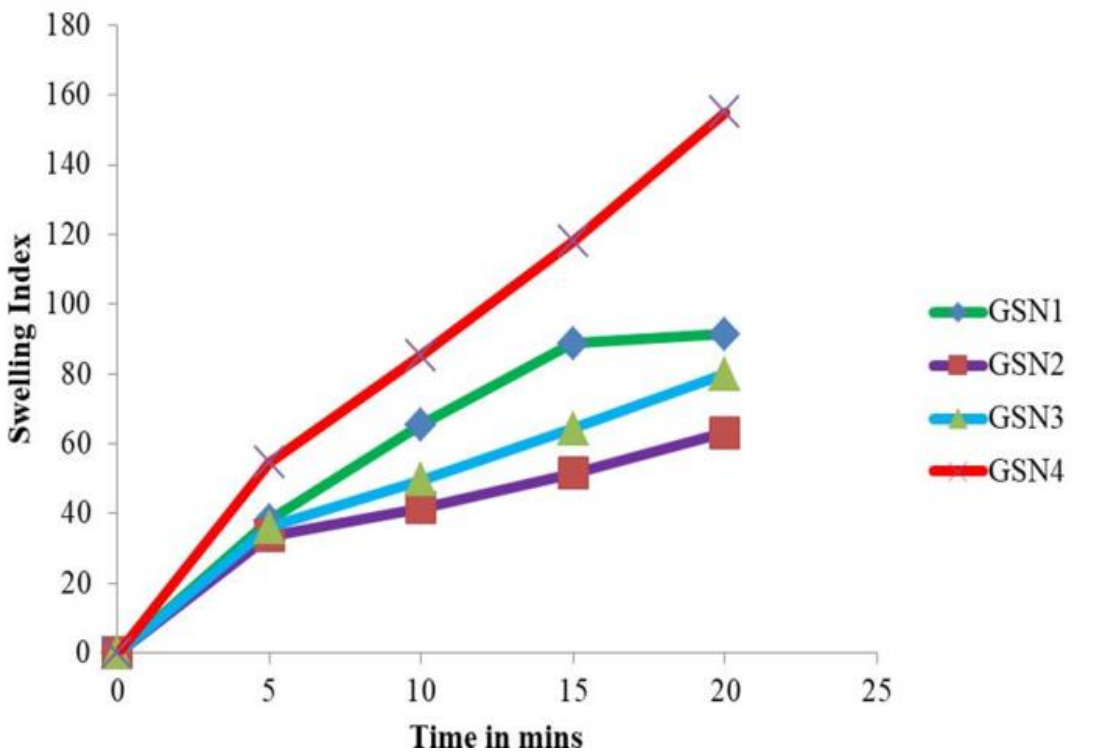
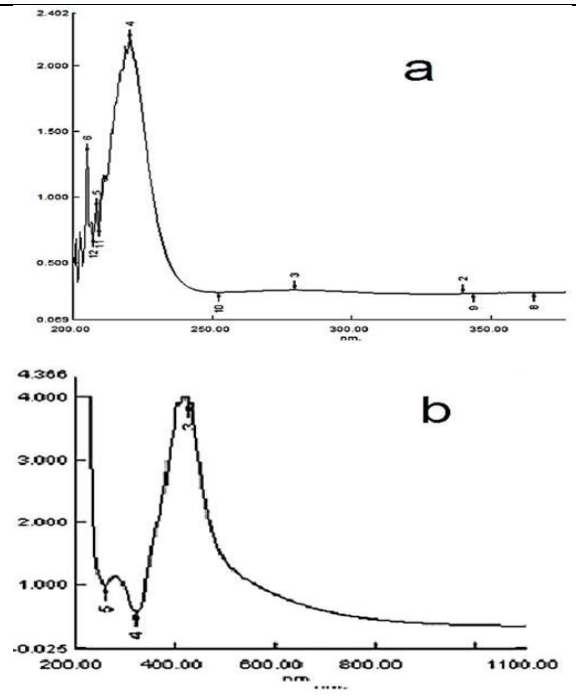


Figure 6: Physical inspection of Silver Nanoparticles.

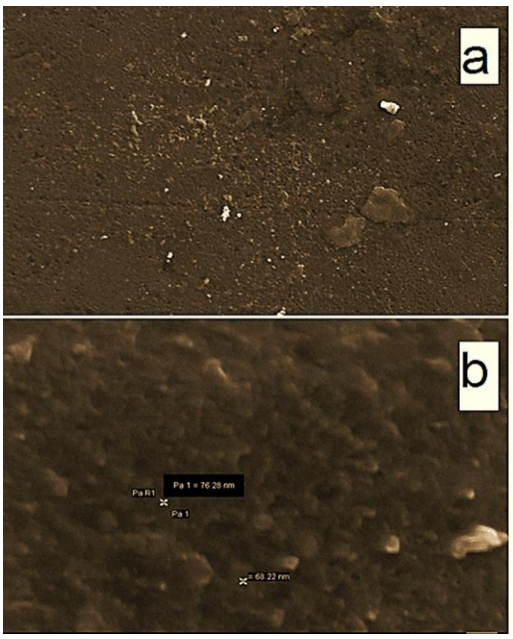
a) Silver nitrate solution b) Produced Silver Nanoparticle Solution

Figure 7: UV- Visible spectrum of Silver Nanoparticles.



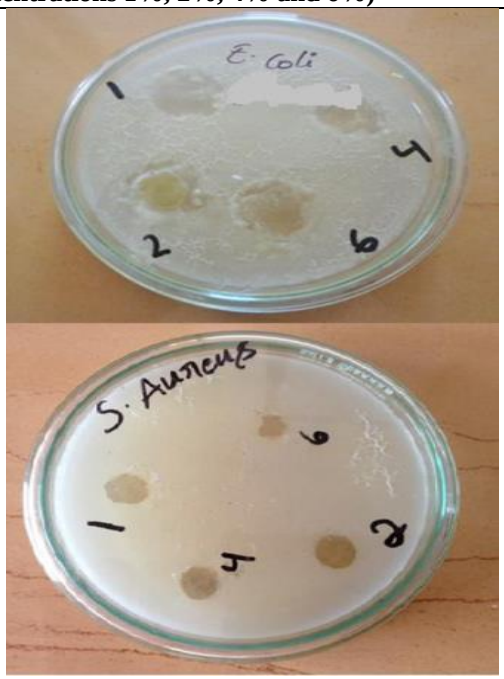
a. Silver nitrate; b. Silver Nanoparticles

Figure 8: SEM images of GSN hydrogel sheet



a. 1.00KX magnification; b. 8.00KX magnification

Figure 9: Antibacterial activity of silver nanoparticles (Concentrations 1%, 2%, 4% and 6%)



DISCUSSION

In the preparation of silver nanoparticles, drop wise addition of sodium citrate to the silver nitrate solution changes the solution colour to bright yellow in colour. The formation of yellow colour is due to the reduction of silver nitrate to silver ions in the presence of sodium citrate as a reducing agent.

FTIR studies confirm the compatibility of silver nanoparticles with the Xanthan and Polyvinyl alcohol. As shown in Figure 6.1-6.3, the spectrum of pure Xanthan film shows a broad band at 3564.89 cm-1 which is due to the OH stretching. The band at 3699.92 cm-1 is assigned for the NH stretching presents the FTIR spectrum of the pure PVA film. The spectrum shows an absorption peak at 3564.89 cm-1 which refers to the intermolecular hydrogen bonding and –OH stretch vibration. The vibrational band observed at 2864.64 cm-1 is associated with the C-H stretching from alkyl groups. The peaks appear at the same wavelength without any change when it is formulated along with silver nanoparticles for the preparation of hydrogel.

Among all the four formulations (GSN1, GSN2, GSN3 and GSN4), GSN4 formulation shows maximum water uptake capacity of 155% and GSN2 formulation shows minimum water uptake capacity of 63% within the time period of 20 minutes.

The prepared Xanthan hydrogel base and silver nanoparticles incorporated Xanthan hydrogel was subjected to scanning electron microscopy (SEM) at different magnifications (1.00, 800 X) to know the surface morphology and particle size of the silver nanoparticles. As shown in the figures, we can confirm the formation of roughly spherical shaped silver nanoparticles with the particle size range of <100 nm.

In UV-VIS spectroscopy, the prepared silver nanoparticles showed an absorption bands at 397 nm and 420 nm as shown in Figure 6.8, which is a typical absorption bands of spherical Ag nanoparticles. The absorption band in visible light region (350 nm – 550 nm, plasmon peak at 420 nm) is typical for silver nanoparticles.

The anti-microbial activity of the silver nanoparticles incorporated Xanthan hydrogel was determined against Staphylococcus aureus (gram positive) and Escherichia coli (gram negative) bacteria. Silver nanoparticles as a broad spectrum antibiotic, inhibits the growth of bacteria with the formation of zone of inhibition in bacteria inoculated nutrient agar medium.

CONCLUSION

Silver nanoparticles with mean diameters of 60-70 nm were synthesized using silver nitrate as a metal precursor and citrate of sodium as a reducing agent. The nanoparticles were characterized by UV/Vis and SEM. UV/Vis spectra show the characteristic surface plasmon absorption peak for the silver nanoparticles ranging from 380 to 410 nm. A successful attempt for the preparation of Xanthan hydrogel was formulated and characterized

with silver nanoparticles. It has proved near optimal requirements for a hydrogel. The prepared hydrogel are promising to give better healing of wound compared to conventional dressing materials. The major advantage is being to provide moist environment for the wound to heal faster. These hydrogel can be utilized for incorporation of wound healing growth factors to fasten the wound healing process and minimize the duration of wound healing.

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